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Formaldehyde as a Potential Human Leukemogen: An Assessment of Biological Plausibility

Robert Golden
ToxLogic, Potomac, Maryland, USA

David Pyatt
Summit Toxicology, Lafayette, Colorado and University of Colorado Schools of Pharmacy and Medicine, Denver, Colorado, USA

Peter G. Shields
Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington, DC, USA

The International Agency for Research on Cancer (IARC, 2004) recently reevaluated the epidemiological data on formaldehyde and concluded that there was "strong but not sufficient evidence for a causal association between leukaemia and occupational exposure to formaldehyde." This conclusion was tempered since a mechanism for leukemia induction could not be identified. Chemically induced leukemia is a well-studied phenomenon with benzene and a number of cancer chemotherapeutic drugs recognized as capable of causing this effect. Abundant in vitro and in vivo data in animals and humans demonstrate that exposure to sufficient doses of these recognized leukemogens can initiate a cascade of events leading to hematopoietic toxicity and the subsequent development of leukemia. This review addresses the biological plausibility that formaldehyde might be capable of causing any type of leukemia by providing a broad overview of the scientific data that must be considered in order to support or refute a conclusion that a particular substance might be leukemogenic. Data on benzene and selected chemotherapeutic cancer drugs are used as examples and are briefly summarized to demonstrate the similar biological events thought to result in leukemogenesis. These data are compared and contrasted with the available data on formaldehyde in order to judge whether they fulfill the criteria of biological plausibility that formaldehyde would be capable of inducing leukemia as suggested by the epidemiological data. Based on the epidemiological data, it is reasonable to expect that if formaldehyde was capable of inducing leukemia, in vivo and in vitro data would offer supporting evidence for biological plausibility. In particular, there is (1) no evidence to suggest that formaldehyde reaches any target organ beyond the site of administration including the bone marrow, (2) no indication that formaldehyde is toxic to the bone marrow/hematopoietic system in in vivo or in vitro studies, and (3) no credible evidence that formaldehyde induces leukemia in experimental animals. As discussed in this review, based on the key biological events that occur in the process of chemically induced leukemia, there is inadequate biological evidence currently available to corroborate existing weak epidemiological associations. This provides an insufficient database to conclude that there is a causal relationship for formaldehyde and leukemia risk.

Keywords Biological Plausibility, Formaldehyde, Leukemia, Leukemogenesis, Mode of Action

I. INTRODUCTION

The International Agency for Research on Cancer (IARC, 2004) recently reevaluated formaldehyde and concluded that two recent studies provided "strong but not sufficient evidence for a causal association between leukaemia and occupational exposure to formaldehyde." The conclusion reached by IARC was based primarily on the observation that "the Working Group could not identify a mechanism for leukaemia induction, and this tempered their interpretation of the epidemiological evidence."

IARC (2004) also concluded that the previously discounted leukemia results reported in seven studies of embalmers, funeral-parlor workers, pathologists, and anatomists, were now

Address correspondence to Robert Golden, ToxLogic, 9808 Clagett Farm Dr., Potomac, MD 20854, USA. E-mail: RGolden124@aol.com

supported by the results of two studies of U.S. industrial workers (i.e., Hauptmann et al., 2003, and Pinkerton et al., 2004). While these epidemiological data form the basis for the "strong but not sufficient" conclusion by IARC (2004), a critical weight-of-evidence evaluation of the epidemiological literature is beyond the scope of this review. However, the results of the most recent studies as well as several critiques of these findings are summarized in the next section.

In order to assess the likelihood that formaldehyde might be leukemogenic, it is necessary to consider the biological basis for leukemogenesis as it is presently understood. That is, what is the biological evidence necessary to conclude that a particular chemical substance is capable of inducing leukemia in either animals or humans? Chemically induced leukemia is a well-studied phenomenon with numerous chemicals demonstrating this capability. For example, abundant in vitro and in vivo data in animals and humans demonstrate that exposure to sufficient doses of benzene can initiate a cascade of events leading to hematopoietic toxicity and the subsequent development of acute mylogenous leukemia (AML). The mechanism(s) responsible for benzene-induced leukemia are not completely understood; however, it has been established that several benzene metabolites may be responsible for bone marrow toxicity (Snyder and Hedii, 1996; Medinsky et al., 1996; Snyder, 2000). The pathway to hematotoxicity and leukemia involves a continuum of events including the likelihood of clastogenic effects from benzene metabolites, perturbations of specific metabolic and detoxification enzymes leading to increased sensitivity or susceptibility of precursor hematopoietic stem cells, and finally interference with regulatory proteins responsible for normal hematopoiesis (U.S. EPA/NCEA, 1997, ATSDR, 1999; Snyder, 2000).

Other chemicals and exposures have also been associated with the induction of leukemia in humans and animals. These include a number of alkylating agents (i.e., cyclophosphamide, chlorambucil, Myleran), topoisomerase inhibitors (i.e., etoposide, teniposide and doxorubicin), and ionizing radiation. All of these leukemogenic exposures exert documented bone marrow toxicity and also demonstrate a range of positive effects in a variety of in vitro tests for hematopoietic toxicity. In other words, all of these substances or exposures share a commonality of biological plausibility as support for their demonstrated leukemogenic properties. A comprehensive review by the U.S. Environmental Protection Agency (EPA) of chemical and radiation-induced leukemogenesis in humans and rodents of many of the same chemicals as considered in the present review (with the notable exception of formaldehyde) confirms the necessity of a general sequence of biological events (U.S. EPA/NCEA, 1997).

IARC (2004) was unable to identify a specific mechanism for leukemia induction as a consequence of exposure to formaldehyde. The lack of corroborating mechanistic data renders the interpretation of the epidemiological evidence somewhat equivocal. Attempting to identify a biologically plausible mode of action would result in one of two likely outcomes:

- A demonstration of biological plausibility for leukemogenesis as a consequence of exposure to formaldehyde would offer compelling and corroborative support for the epidemiological findings.
- A demonstration that it is biologically implausible that leukemia can be caused by formaldehyde would suggest that the epidemiological findings were either incorrect, confounded, or spurious.

Consequently, a critical review of the biological plausibility that formaldehyde might be capable of causing leukemia is likely to either support or refute the epidemiological findings. This review is intended to provide a broad overview of the scientific data that must be considered in order to support or reject a conclusion that a particular substance might be capable of inducing leukemia. Data on benzene and selected chemotherapeutic cancer drugs are used as examples and summarized with enough detail to demonstrate the general consistency of biological events leading to leukemogenesis. These data are then compared and contrasted with the available data on formaldehyde in order to judge whether they fulfill the criteria of biological plausibility that formaldehyde would be capable of inducing leukemia as suggested by the epidemiological data. The comparative approach as just outlined was taken, rather than a formal weight-of-evidence analysis using mode-of-action data as detailed in the U.S. EPA recently revised cancer risk assessment guidelines (U.S. EPA, 2005). These guidelines lay out a detailed framework for establishing the mode of action of an individual chemical. As described later, given the lack of any experimental data suggesting that formaldehyde might have leukemogenic properties, the only way to assess these data in the context of leukemogenesis was in comparison with the mode of action of known leukemogenic substances.

II. OVERVIEW OF RECENT EPIDEMIOLOGICAL FUNDINGS AND CRITIQUES CONCERNING REPORTED ASSOCIATION BETWEEN FORMALDEHYDE AND LEUKEMIA

The study by Hauptmann et al. (2003) consisted of a cohort of 25,619 industrial workers at 10 U.S. industrial plants where formaldehyde was either produced, or used in the production of other products. Formaldehyde exposure was assessed by peak, average intensity, cumulative, and duration. Compared with workers exposed to low peak levels of formaldehyde (0.1–1.9 ppm), relative risks for leukemia (particularly myeloid leukemia) were 2.43 (95% CI = 0.81–7.25) and 3.46 (95% CI = 1.27–9.43) for workers exposed to peak levels of 2.0–3.9 ppm and ≥4.0 ppm, respectively. Compared with workers exposed to low levels of average exposure intensity of formaldehyde (0.1–0.4 ppm), workers exposed to 0.5–0.9 ppm and ≥1.0 ppm average intensity had relative risks of 1.15 (95% CI = 0.41–3.23) and 2.49 (95% CI = 1.03–6.03), respectively. The relative risk for leukemia was not significantly associated with cumulative exposure or with duration of exposure.

Using the original data from Hauptmann et al. (2003), this cohort has been reanalyzed by Marsh and Youk (2004). The U.S. and local county rate-based standardized mortality ratios (SMRs) and relative risks (RR) of leukemia and myeloid leukemia (ML) were recomputed by the same four categories of formaldehyde exposure metrics as used by Hauptmann et al. (2003), in addition to an alternative categorization based on tertiles of deaths from all leukemia among exposed subjects. This analysis revealed that the elevated RR for all types of leukemia combined and for ML RRs and associated trends reported by Hauptmann et al. (2003) for highest peak and average intensity of formaldehyde exposure categories occurred because null (or slight) to moderate mortality excesses were compared with statistically significant baseline deficits in deaths from these diseases in the internal comparison group. The alternative categorization based on average intensity of exposure yielded leukemia and ML SMRs close to 1.0 in the highest exposure category, and also demonstrated less evidence of a trend in RRs for leukemia and ML. Similar to the findings of Hauptmann et al. (2003), there was no association for cumulative and duration of formaldehyde exposure as well as no consistent evidence that leukemia or ML risks increased with increasing duration of time spent in a given highest peak exposure. This reanalysis, therefore, did not support the conclusions reached by Hauptmann et al. (2003) that a causal association between formaldehyde exposure and increased mortality from leukemia and ML exists.

In the study by Pinkerton et al. (2004), the mortality experience of 11,039 garment workers exposed to formaldehyde for 3 months or more at three plants was evaluated. While noting that the mean time-weighted average formaldehyde exposure at the three plants in the early 1980s was 0.15 ppm and that past exposures may have been substantially higher, no individual formaldehyde exposure measurements were available. Compared to U.S. mortality rates, in the total cohort, mortality from myeloid leukemia was not significantly increased (SMR = 1.44, 95% CI 0.80–2.37). Mortality from myeloid leukemia was greatest among workers first exposed in the earliest years, when exposures were presumably higher. Among workers with both 10 years or more of exposure and 20 years or more since first exposure, mortality from leukemia and myeloid leukemia were significantly increased (SMR = 1.92, 95% CI 1.08–3.17) and (SMR = 2.55, 95% CI 1.10–5.03), respectively.

In another recent study of a cohort of 14,014 men employed after 1937 at six British factories where formaldehyde was produced or used, there was no increased mortality from leukemia relative to the national population even in those exposed at 2 ppm or greater (SMR = 0.71, 95% CI 0.31–1.39) (Coggon et al., 2003).

In a letter to the editor, Casanova et al. (2004) raised the issue of the lower than expected mortality from lymphohematopoietic disease (SMR = 0.6, 95% CI 0.4–0.7) and leukemia (SMR = 0.5, 95% CI 0.28–0.8) in the referent group (<2 ppm) as the basis for the findings of Hauptmann et al. (2003). Also noted was the lack of a significant association with all lymphohematopoietic neoplasms in formaldehyde-exposed workers in comparison with an external comparison group (SMR = 0.8, 95% CI 0.7–0.9). In response, Hauptmann et al. (2004) disagreed that external comparisons were appropriate and that other workers were the preferred comparison group, although they did not directly address the consequences of a deficit in lymphohematopoietic neoplasms in the internal comparison group. They also reiterated that the increasing risk with increasing exposure as originally reported was an important element in support of an exposure-response relationship.

Cole and Axten (2004) have also critically evaluated the epidemiological data supporting the conclusion that a causal association between leukemia and exposure to formaldehyde exists. This review considered the recent studies by Hauptmann et al. (2003), Coggon et al. (2003), and Pinkerton et al. (2004), as well as previous studies in the context of the established causation criteria, that is, consistency, strength of association, coherence, dose-response, and biological plausibility. The authors concluded, "In sum, then, the formaldehyde-leukemia hypothesis fails each of the four guidelines of general causation. This is hardly surprising in view of the weak and inconsistent findings in the most recent epidemiologic research and the consistent findings in animal studies."

As described earlier, particularly the results of the Hauptmann et al. (2003) study on increased mortality risks from leukemia in the large National Cancer Institute (NCI) formaldehyde cohort study have generated controversy pertaining to the validity of the reported findings. Because these studies are complicated, there are legitimate grounds for differences of opinion on how the data are interpreted. However, the consistency of the skepticism is noteworthy. Even though the NCI study was published in 2004, NCI has already agreed to undertake an update of their study, which will add an additional 8 years of already available data to the evidence. This update should confirm or refute whether exposure to formaldehyde is associated with increased risk of cancer.

III. BENZENE

Benzene was first identified as a human carcinogen as a consequence of a clear causal association between occupational exposure and the development of acute myelogenous leukemia (AML) in humans following long-term exposure (Aksoy, 1989; Infante et al., 1977; NTP 1994; IARC, 1987). Paradoxically, however, despite abundant animal data confirming the carcinogenicity of benzene (e.g., Zymbal gland carcinoma, skin, lymphoma, mammary carcinoma, etc.) (e.g., Huff et al., 1989), early studies with benzene were unable to confirm its leukemogenic properties as observed in humans. Cronkite et al. (1984) reported a highly significant increase in thymic and nonthymic lymphomas in C57BL/6 mice exposed to 300 ppm of benzene by inhalation 6 h/day, 5 days/week for 16 weeks. In a continuation of that study (Cronkite et al., 1985), a definite pattern for thymic and nonthymic lymphoma appearance and mortality was observed. While the underlying reasons are

not clear, lymphomas/lymphatic leukemias are the predominant form of benzene-induced hematological neoplasia in rodents. Clear species specificity exists between rodents and humans, as acute myeloid leukemia is the only malignancy associated with benzene exposure in humans.

Several additional studies have shown benzene to be leukemogenic in rodents following inhalation exposure, thereby providing an animal model for more detailed study of potential modes of action. In a study by Snyder et al. (1984), Sprague-Dawley rats exposed to 100 ppm benzene for 6 h/day, 5 days/week for a lifetime developed myelogenous leukemia and liver tumors. In a series of studies (Cronkite, 1986; Cronkite et al., 1984, 1985), C57BL/6 and CBA/Ca mice were exposed to 300 ppm benzene by inhalation 6 h/day, 5 days/week for 16 weeks. These mouse strains were used because of their susceptibilities to ionizing radiation-induced thymic lymphoma and also for their low spontaneous rates of AML. CBA/Ca male mice exposed to 100 ppm of benzene 6 h/day, 5 days/week for 16 weeks developed mylogenous leukemia, while C57BL/6 mice similarly exposed to 300 ppm had a significant increase in the incidence of thymic and nonthymic lymphomas (Cronkite, 1986; Cronkite et al., 1989). Increased incidences of Harderian and Zymbal gland, squamous-cell, and mammary carcinoma, papilloma, and adenocarcinoma of lungs were also seen. The responses of rodents and humans to chronic benzene exposure are not the same particularly with regard to leukemia induction. Nonetheless, myeloproliferative disorders following benzene exposure in rodents have been used with varying degrees of success to investigate benzene-induced leukemia.

Studying the influence of benzene on the hematopoietic system in rodents has provided some useful insights into the potential mode of action. In female BDF_1 mice, benzene inhalation exposure at 100, 300, and 900 ppm for 6 h/day, 5 days/week for 8 weeks produced pronounced effects on erythroid committed bone marrow progenitor cells as measured by various in vitro culture assays (erythroid burst-forming unit [BFU-E] and erythroid colony-forming unit [CFU-E] assays; Seidel et al., 1989). Farris et al. (1997) conducted an inhalation study in male B6C3F1 mice exposed to 1, 5, 10, 100, and 200 ppm benzene for 6 h/day, 5 days/week for 1, 2, 4, or 8 weeks. While there were no significant effects on hematopoietic parameters below 10 ppm, 100 and 200 ppm reduced the number of total bone marrow cells, progenitor cells, differentiating hematopoietic cells, and most peripheral blood parameters. In addition, replication of bone-marrow-derived hematopoietic progenitor (HPC) cells was increased during the exposure period as likely compensation for the cytotoxicity induced by 100 and 200 ppm benzene. In a similar study, male B6C3F1 mice were exposed to 0, 1, 10, 100, or 200 ppm benzene by inhalation for 6 h/day, 5 days/week, for 1, 2, 4, or 8 weeks, with evaluations of primitive and committed progenitor cells, differentiating and maturing lineage-specific cells, and stromal cells in the bone marrow at each sampling time. At 100 and 200 ppm there were rapid and significant reductions in number of reticulocytes in the blood, B lymphocytes in the bone marrow and spleen, and an increased frequency of micronucleated reticulocytes in the bone marrow, thus demonstrating substantial hematopoietic toxicity (Farris et al., 1996).

In an in vivo/in vitro study, mice were exposed to 300 ppm benzene for 6 h/day, 5 days/week for 2 weeks, followed by growth of bone marrow cells grown in long-term bone marrow culture. Bone marrow cultures initiated 1 day after the last benzene exposure did not produce adequate numbers of hematopoietic cells over 3 weeks, and, in most cases, no erythroid or myeloid clonogenic were recovered. These results clearly demonstrate the bone marrow target organ specificity of benzene exposure (Abraham, 1996). Numerous other in vivo and in vitro studies attest to the effects of benzene on bone-marrow-derived hematopoietic stem and progenitor cell differentiation (Irons and Stillman, 1996b; Niculescu and Kalf, 1995) and gene expression profiles in bone marrow and hematopoietic stem cells (Faiola et al., 2004). In addition, several hypotheses regarding potential modes of leukemogenic action of benzene have been published, including cell cycle suppression in hematopoietic progenitor and stem cells and selective chromosomal aberrations in bone marrow cells (Yoon et al., 2001; Hsieh et al., 1999; Stillman et al., 2000; Irons and Stillman, 1996a; Parke, 1996; Snyder and Hedii, 1996). Consequently, it is reasonable to conclude that leukemogenic transformation induced by benzene involves damage to the bone marrow and a resulting dysregulation of hematopoiesis.

IV. CANCER CHEMOTHERAPEUTIC DRUGS AND OTHER EXPOSURES AS LEUKEMOGENIC SUBSTANCES

A. Alkylating Agents

It has been generally recognized that treatment of primary malignancies with cytotoxic drugs that act as alkylating agents can lead to myelodysplastic syndrome (MDS) and/or acute myelogenous leukemia (Jandl, 1997). This list includes, but is not limited to, melphalan, chlorambucil, busulfan, cyclophosphamide, and nitrosourea (IARC, 1987). Since most modern therapeutic regimens utilize a combination of drugs, it is often difficult to discern the precise offending agent. Nonetheless, as a class, there can be little doubt that treatment with these drugs alone or in various chemical "cocktails" increases the risk of developing secondary AML (s-AML). Secondary leukemias have been estimated to account for 10–30% of all AML (Leone, 1999). The exact risk is not known with certainty and will likely vary considerably depending on treatment and primary disease (Pui, 1991; Pedersen-Bjergaard, 1985; Brusamolina, 1998)

It is also clear that AML arising secondary to treatment with alkylating chemotherapeutic agents often possesses morphological and cytogenetic characteristics that can be used to distinguish it from AML arising de novo, or primary, which has no readily identifiable cause in most patients (Coltman and Dahlberg, 1990; Park and Koeffler, 1996). This includes disease progression and the presence of specific cytogenetic

abnormalities (Jandl, 1997; Leone et al., 1999; Pedersen-Bjergaard et al., 1985, 2002). As the disease progresses, cytogenetic abnormalities are observed in virtually every case of s-AML, providing evidence of the genotoxic mechanism involved in the origin of the disease (Jandl, 1997, Leone et al., 1999; Linet et al., 1996; Snyder and Kalf, 1994).

All of the cytotoxic alkylating chemotherapeutic drugs that cause s-AML display damaging effects on the bone marrow. Because bone marrow is an organ with rapid cell growth, the hematopoietic toxicity of cytotoxic agents is a consequence of the very property for which they are used clinically, that is, to kill rapidly growing cancer cells. Confirmatory of their leukemogenic potential, numerous epidemiological studies of patients receiving a variety of such drugs have shown associations with leukemia in addition to other types of cancer (e.g., bladder cancer). For many of these drugs, their leukemogenic potential has also been confirmed in experimental animal studies, as well as in in vitro studies demonstrating bone marrow toxicity. While it is beyond the scope of this review to consider in detail the volume of data on this complex issue, some of the relevant data on a few cancer chemotherapeutic drugs associated with leukemia are described in order to illustrate the point that their potential to cause leukemia in humans is supported by concordant in vivo and/or in vitro data showing a similar potential. However, unlike the extensive database for benzene, including detailed studies on a likely mode of action, the animal data for these cancer chemotherapeutic drugs are far less robust. Nevertheless, these data reinforce the idea that to conclude that it is biologically plausible that any particular substance might be capable of causing leukemia requires that certain basic criteria be satisfied (U.S. EPA/NCEA, 1997).

It should be noted that x-ray and γ radiation also unequivocally cause leukemia in animals and humans, and also demonstrate considerable bone marrow/hematopoietic toxicity in both in vivo and in vitro systems (IARC, 2000; U.S. EPA/NCEA, 1997). However, these exposures are not included in this review due to the fact that unlike chemicals, which must be absorbed and distributed via the circulation to the bone marrow in order to induce leukemogeic effects, radiation-induced leukemogenesis with penetration through the body does not involve this critical step.

1. Cyclophosphamide

Cyclophosphamide is probably the most studied of the cancer chemotherapeutic drugs with an established ability to cause secondary human leukemia (IARC, 1987). For example, among 602 patients treated predominantly with cyclophosphamide for non-Hodgkin's lymphoma in Denmark, 9 cases of acute nonlymphocytic leukemia (ANLL) or preleukemia (i.e., MDS) were observed, compared to 0.12 expected on the basis of incidence rates in the general population (Pedersen-Bjergaard et al., 1985). The finding of preleukemia (i.e., MDS) is highly indicative of frank bone marrow insult. In the United States, 3 three cases of ANLL or preleukemia were observed among 333 women treated only with cyclophosphamide for ovarian cancer, while 1.2 cases were expected (Greene et al., 1986). In Germany, a case-control study of leukemia arising as a second primary malignancy following breast or ovarian cancer was reported by Haas et al. (1987). Relative risks of 1.5, 3.3, and 7.3 were estimated in association with cumulative doses of <10 g, 10–29 g, and >30 g cyclophosphamide, respectively.

In numerous short-term in vivo assays in mice, cyclophosphamide demonstrates substantial dose-related effects on pluripotent and committed stem-cell colony-forming-unit assays (CFU-S and CFU-C). Similar effects have also been reported in assays conducted with human stem cells. Some of these effects have been reversible after cessation of dosing. Repeated or chronic administration of cyclophosphamide has also produced various dose-related adverse effects on hematopoietic stem cells. In humans, clinical administration of cyclophosphamide has produced severe depression of peripheral white blood cells (WBC), that is, pancytopenia. Doses had to be reduced or discontinued after more than 4 months due to increasing sensitivity of the granulopoietic system to the drug, suggesting cumulative toxicity (Lohrmann and Schreml, 1982).

Lifetime oral administration of low doses of cyclophosphamide to Sprague-Dawley rats produced malignant tumors in lymphoid and hematopoietic tissues, in addition to other organs (Schmahl and Habs, 1978). Doses were administered 5 days/week in drinking water. Of interest was the finding that while the highest dose (2.5 mg/kg/day) produced a clear carcinogenic effect in hematopoietic tissue over controls, lower doses (0.31–1.25 mg/kg/day) produced a greater effect. In a study designed to investigate the extent to which the induction of leukemia by cyclophosphamide might be influenced by genetic predisposition, this drug was administered sc at 13 and 26 mg/kg weekly for a lifetime to AKR mice, which are genetically predisposed to develop leukemias, and to NMRI mice, which exhibit a low spontaneous leukemia rate. In AKR mice, cyclophosphamide decreased the incidence of leukemias by 17% and 37%, respectively, while in NMRI mice, cyclophosphamide significantly increased the incidence of leukemias by 46% at the low dose and 26% at the high dose (Petru et al., 1989). The effects of daily sc administration of cyclophosphamide to female NZB/NZW mice at 1 or 8 mg/kg was reported by Walker and Bole (1971). Six of 10 high-dose animals developed leukemias and other malignancies after 36 to 64 weeks of treatment. These findings support the leukemogenic potential of cyclophosphamide.

Genotoxicity data in humans have demonstrated increased incidence of sister chromatid exchanges in peripheral blood lymphocytes and, in one study, in bone marrow cells of patients treated with cyclophosphamide for a variety of malignant and nonmalignant diseases (IARC, 1987). While consistently positive results have also been reported when cyclophosphamide has been tested for genetic effects in a wide variety of in vivo

and in vitro tests, all of these findings are nonspecific and not confirmatory of leukemogenic potential.

The totality of the data on cyclophosphamide indicates that it is a carcinogen with the bone marrow as one of its primary target organs. This is evidenced by the induction of leukemia in both animals and humans as well as multiple in vitro short-term and in vivo chronic studies. Taken collectively, these data support clinical evidence for its leukemogenic potential.

2. 1,4-Butanediol Dimethanesulfonate (Myleran)

According to the IARC (1987), there is sufficient evidence to conclude that Myleran is carcinogenic in humans. In a study of 69 patients with bronchial carcinoma who had been treated with Myleran and survived for 5 years, 4 developed acute nonlymphocytic leukemia (3 myelomonocytic leukemias and 1 erythroleukemia) and 15 others developed, pancytopenia in the succeeding 4 years. In contrast, among 148 other survivors at 5 years who had not been given Myleran, 1 case of pancytopenia was reported (Stott et al., 1976). Stott et al. (1976) reported the 5-year findings of a double-blind study following long-term chemotherapy with Myleran or cyclophosphamide for carcinoma of the bronchus compared with a group receiving a placebo. Hematological toxicity, especially thrombocytopenia, was frequent and severe in the patients who were treated with Myleran, and low platelet counts continued long after chemotherapy was discontinued.

In animals, Myleran has been tested for carcinogenicity by intraperitoneal (ip) injection and by intravenous (iv) injection in mice and rats and by oral administration to rats with both positive and negative findings. Administration of Myleran to mice (ip) did not increase the incidence of tumors in two studies (IARC, 1974; Stoner et al., 1973). However, leukemia and hypoplastic bone marrow were reported in two other studies (Chu et al., 1981; Morley and Blake, 1974).

In numerous short-term in vivo assays in mice, Myleran demonstrates substantial doserelated effects on hematopoietic proliferation and differentiation (CFU-S and CFU-C assays). Similar effects have also been reported in assays conducted in dogs with a dose-dependent reduction of CFU-C. These effects have generally been reversible after cessation of dosing, although, depending on the dose and particular assay, recovery may be slow. Repeated or chronic administration of Myleran has also produced various dose-related adverse effects on hematopoietic progenitor cells, with the most prominent effects on the least mature cells among hematopoietic progenitor cells. Additional studies suggest that hematopoietic failure may be a consequence following sufficient doses of Myleran, which produces a long-term inability of stromal cells to reproduce and support normal hematopoiesis (Lohrmann and Schreml, 1982; Guest and Uetrecht, 2000; Trainor and Morley, 1976; Dunn and Elson, 1970).

Chronic treatment of rodents with Myleran in vivo induced dominant lethal mutations and increased the frequency of chromosomal aberrations and micronuclei in bone marrow cells; in single studies, Myleran induced DNA damage but not mutation. Myleran is genotoxic, as shown by its ability to induce chromosomal aberrations and sister chromatid exchanges in human and rodent cells in vitro and mutation in rodent cells in vitro (IARC, 1987), although these findings are nonspecific and not confirmatory of leukemogenic potential.

The totality of the data on Myleran indicates that it is a carcinogen with the bone marrow as one of its primary target organs. This is evidenced by the induction of leukemia in both animals and humans as well as multiple in vitro short-term and in vivo chronic studies. Taken collectively, these data support clinical evidence for its leukemogenic potential.

3. Chlorambucil

According to the IARC (1987), there is sufficient evidence to conclude that chlorambucil is carcinogenic in humans. Chlorambucil is an alkylating chemotherapeutic drug used for the treatment of cancer (i.e., breast and ovarian) as well as other noncancer diseases such as juvenile arthritis and glomerulonephritis. While the studies demonstrating the carcinogenicity of chlorambucil are small and in some cases involve simultaneous exposure to radiation or other potential carcinogens, all report an excess of subsequent malignancy, particularly acute nonlymphocytic leukemia (ANLL) (IARC, 1981; Green et al., 1982). Berk et al. (1981) reported a 13-fold increase in the incidence of ANLL in 431 polycythemia vera patients receiving chlorambucil therapy. The incidence of ANLL was 2.3 times higher than in patients receiving radioactive phosphorus, with the excess strongly related to the dose of chlorambucil. Reimer et al. (1977) reported on acute leukemia following the use of a variety of alkylating agents (e.g., cyclophosphamide, chlorambucil, etc.) for the treatment of ovarian cancer. Thirteen cases of ANLL occurred among 5455 patients compared to 0.62 cases expected (RR = 21.09). Similar long-term follow-up studies of patients treated for a variety of cancers with alkylating agents have also reported increased incidence of leukemia (Petru and Schmahl, 1991).

In animals, chlorambucil has been tested for carcinogenicity in mice and rats by ip injection and in female rats by oral gavage. It produced tumors of the lung, hematopoietic system and ovaries in mice (IARC, 1981), and hematopoietic tumors in male rats and hematopoietic and lymphatic tumors in female rats (IARC, 1981; Berger et al., 1985; Weisburger, 1977).

Chlorambucil also produces residual bone marrow toxicity in mice following exposure as measured by CFU-S, CFU-C, and significant reductions in tibeal bone marrow cellularity (Trainor et al., 1979; Van Putten and Lelieveld, 1971). Valeriote and Tolen (1972) reported decreased survival of hematopoietic colony-forming cells in vivo following administration of chlorambucil. Chlorambucil is genotoxic, as demonstrated by its ability to induce sister chromatid exchanges and chromosomal aberrations in human lymphocytes, sister chromatid exchanges and mutation in Chinese hamster cells in vitro and mutations in bacterial test

systems (IARC, 1987), although these findings are nonspecific and not confirmatory of leukemogenic potential.

The totality of the data on chlorambucil demonstrates that it is a carcinogen with the bone marrow as one of its primary target organs, as evidenced by the induction of leukemia in both animals and humans. In vivo studies also demonstrate that the hematopoietic system (i.e., bone marrow) is a target organ for chlorambucil-induced adverse effects, thus confirming its leukemogenic potential.

B. Topoisomerase Inhibitors

Recently, clinical studies have revealed that a different form of AML can arise secondary to treatment with drugs that primarily target topoisomerase II, an enzyme required for DNA replication, recombination, and repair (Beaumont et al., 2003; Hoffman et al., 1995; Anderson et al., 2002; De Renzo et al., 1999; Pedersen-Bjergaard et al., 2002). Etoposide, teniposide, and other epipodophyllotoxins as well as anthracycline-based antibiotics such as doxorubicin have been implicated in the etiology of this form of secondary leukemia (Beaumont et al., 2003; U.S. EPA/NCEA, 1997; De Renzo et al., 1999). Leukemia secondary to treatment with topoisomerase inhibitors presents with a distinct clinical picture compared to secondary leukemia associated with high-dose therapy with alkylating agents. Leukemia secondary to topoisomerase II inhibition or radiation will often have a shorter latency (6–36 months) and will lack evidence of a preceding myelodysplasia (Beaumont et al., 2003; Bowen, 2000). Further, cytogenetic lesions associated with t-AML following exposure to topoisomerase inhibitors are often the same as reported in de novo leukemia (De Renzo et al., 1999; Pedersen-Bjergaard et al., 2002).

Because these drugs are relatively new, there is not a robust animal database as with the alkylating agents, particularly with respect to cancer bioassays. However, in studies with mice, the topoisomerase inhibitor bimolane (ICRF 159) produced a dose-related increase in lymphocytic leukemia in female mice and none in male mice. In another study, bimolane produced granulocytic leukemia in mice (U.S. EPA/NCEA, 1997). Etoposide induces DNA damage in rat bone marrow cells (Cierniak et al., 2004) as well as in mouse bone marrow (chromosomal aberrations, increase in mitotic index and micronucleus; Choudhury et al., 2004; Attia et al., 2003). In addition, etoposide has produced considerable myelotoxicity in humans following its use in various chemotherapy regimens (Bar-Sela et al., 2003). Similarly, teniposide produces micronuclei in mouse bone marrow (Jagetia and Aruna, 1999), in addition to severe myelotoxicity and aplastic bone marrow in humans following treatment for various types of cancer (Cascinu et al., 1997; Smit et al., 1992; Ochs et al., 1991). Both etoposide and teniposide are also mutagenic (Nakanomyo et al., 1986). Doxorubicin produces bone marrow toxicity in vitro (Lin et al., 2004) as well as in vivo in mice (Oredipe et al., 2003) and rats (To et al., 2003).

The totality of the data on topoisomerase inhibitors indicates that members of this class of chemotherapeutic drugs are carcinogens with the bone marrow as one of the primary target organs. This is evidenced by the induction of leukemia in both animals and humans, as well as in vitro and in vivo data demonstrating bone marrow toxicity. Taken collectively, these data support clinical evidence for their leukemogenic potential.

C. Smoking

The relationship between cigarette smoking and increased risk of leukemia has generated considerable debate, but now smoking is generally considered a weak leukemogen. In 1979, the Surgeon General reported that smoking is a major cause or contributing factor in a variety of cancers, but did not list leukemia among them. However, many of the studies evaluated in that report did show an elevated risk of developing leukemia, but no dose response was discernable. Nonetheless, Austin and Cole (1986) suggested that there may be a causative link, especially with AML. This was a highly provocative suggestion for several reasons, not the least of which is that benzene is found and produced in cigarette smoke. As a result, there have been several follow-up studies, with mostly inconclusive findings. Some studies have reported increases in AML as well as other forms of leukemia, some have only seen increases in all types of leukemia combined, and many have been negative (Severson et al., 1990; Brownson, 1989; McLaughlin et al., 1989; Heath, 1990). Part of the problem is that the relative risk of developing AML from smoking is ~1.5 (as reported in most studies). Therefore, depending on the population size, a study could report this to be significantly elevated or not. However, in 1993, a meta-analysis was conducted that provided the single best evidence for a causative link between smoking and AML (or ANLL, acute nonlymphocytic leukemia, as it is sometimes referred to) (Brownson et al., 1993). As previously mentioned, the presence of benzene or benzene metabolites such as hydroquinone and phenol adds considerable biological plausibility to this hypothesis. In heavy smokers, the absolute dose of benzene, accumulated over a lifetime, is not trivial. Modeled estimates of the potential contribution of benzene to smoking-related risk of leukemia suggest that benzene could be responsible for approximately one-tenth to one-half of smoking-induced total leukemia mortality and up to three-fifths of smoking-related AML mortality (Korte et al., 2000). However, it must be emphasized that cigarette smoke is a highly complex mixture of numerous potential carcinogens, so that while one component (i.e., benzene) can be modeled with the hypothesis that benzene within cigarette smoke plays an etiological role in the development of leukemia, the leukemogenic effects could be due to other carcinogens. Parenthetically, although trace levels of formaldehyde are also found in cigarette smoke, there is insufficient evidence to implicate this exposure in smoking-related leukemia.

V. FORMALDEHYDE

In keeping with the demonstrated bone marrow/hematopoietic toxicity of benzene and several cancer chemotherapeutic drugs, multiple lines of evidence must be

considered in order to support the biological plausibility that exposure to formaldehyde could also cause the development of leukemia. Central to this issue is the ability to demonstrate (1) that inhaled or ingested formaldehyde can reach the bone marrow (i.e., target organ), (2) that formaldehyde which reaches the bone marrow can produce hematopoietic toxicity, and (3) that there is evidence in animal studies that exposure to formaldehyde is capable of inducing a leukemogenic response. An inability to fulfill these biologic plausibility requirements of leukemogenesis would demonstrate either (a) that formaldehyde acts through a unique and unknown mode of action or, more likely, (b) that formaldehyde is not leukemogenic, suggesting that the epidemiological findings were either incorrect or not due to formaldehyde (i.e., confounded).

A. Potential for Hematopoietic (i.e., Distant Site) Toxicity

Formaldehyde is a highly reactive substance that likely exerts its corrosive and cytotoxic effects due to its ability to readily combine with free, unprotonated amino groups of amino acids or DNA to yield hydroxymethyl amino acid derivatives and a proton (H^+). It is likely that formaldehyde toxicity occurs when intracellular levels saturate formaldehyde dehydrogenase and other metabolic detoxification activity, thereby overwhelming the natural protection against formaldehyde-induced toxicity. This would then permit unmetabolized formaldehyde to exert adverse effects locally. As shown in Figure 1, the primary metabolite of formaldehyde is formate. This reaction is catalyzed by cytosolic glutathione (GSH)-dependent formaldehyde dehydrogenase (FDH), for which GSH is required as a cofactor. The reaction of formaldehyde with GSH yields *S*-hydroxymethylgluthatione (GSH conjugate) which in the presence of NAD^+ and FDH forms the thiol ester of formic acid via the action of *S*-formylgluthathione hydrolase (SFGH). Formic acid is not as reactive as formaldehyde itself and can either enter into the one-carbon metabolic pool for incorporation into other cellular components, be excreted as a salt in the urine, or be further metabolized to carbon dioxide (ATSDR, 1999).

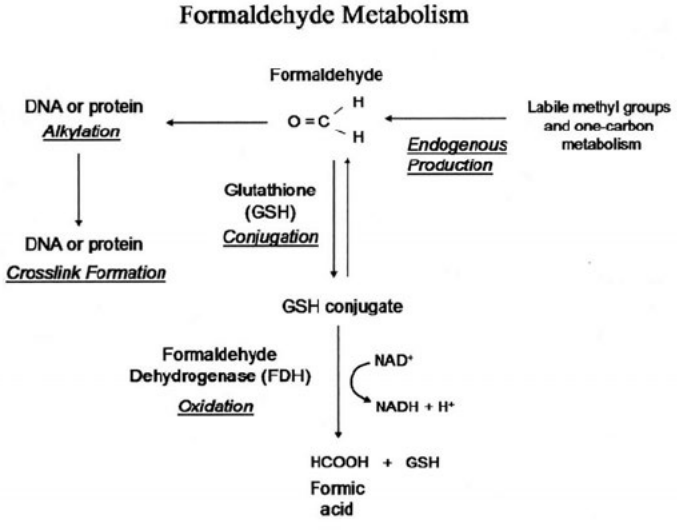

FIG. 1. Primarily metabolic pathway of formaldehyde biotransformation.

This general sequence of events shown in Figure 1 is supported by a number of studies in rodents, monkeys, and humans suggesting that if exposure levels of formaldehyde are below concentrations that can be rapidly metabolized by tissue formaldehyde dehydrogenase and other detoxification enzymes, blood levels do not appreciably increase. As noted in ATSDR (1999):

> "The lack of toxicity is likely related to rapid metabolism prior to the formaldehyde reaching the blood and blood-forming components (bone marrow). Some evidence suggests, however, that the rapid metabolic capabilities can be overwhelmed to some degree (Vargova et al., 1993), resulting in some minor alterations in blood parameters. In that study, affected male rats received a gavage dose level of 80 mg/kg/day formaldehyde for 4 weeks. This dosing method may have resulted in large doses of formaldehyde being absorbed over a shorter period of time than in the drinking water studies. In this situation, some unmetabolized formaldehyde may have been responsible for the alterations in erythrocyte count and hemoglobin and mean cellular hemoglobin values. (p.)

Heck et al. (1985) determined the effect of exposure to formaldehyde on the concentration in the blood in rats and humans. Following exposure of 8 male F-344 rats to 14.4 ppm of formaldehyde for 2 hours, the blood was collected immediately after exposure. Blood from eight unexposed rats served as controls. Analysis by gas chromatography/mass spectrometry (GCMS) showed formaldehyde concentrations of 2.24 ± 0.07 and 2.25 ± 0.07 μg/g blood in exposed rats and controls, respectively. Formaldehyde concentrations in human venous blood from four males and two females were determined by analyzing blood samples collected before and after exposure to 1.9 ppm formaldehyde for 40 min. Average formaldehyde concentrations before and after exposure were 2.61 ± 0.14 and 2.77 ± 0.28 μg/g blood, respectively. In neither rats nor humans was there a statistically significant effect of formaldehyde exposure on the average concentrations in the blood.

In a similar study, 3 rhesus monkeys were exposed to formaldehyde at 6 ppm, 6 h/day, 5 days/week for 4 weeks and the formaldehyde concentration in the blood was measured by gas chromatography mass spectroscopy (GCMS). The formaldehyde concentrations immediately after the final exposure in the 3 exposed and 3 unexposed animals were 1.84 and 2.42 μg/g blood, respectively. Additionally, after a further 45 h without exposure to formaldehyde, blood concentrations did not differ significantly. These results demonstrate that subchronic inhalation exposure of nonhuman primates to formaldehyde has no significant effect on the concentration in the blood, and that the average concentration of formaldehyde in the blood of monkeys is similar to that observed in human studies (Casanova et al., 1988).

In order to further explore these issues, [^{14}C]- and [^{3}H] formaldehyde was studied for its ability to label macromolecules

(i.e., DNA, RNA, and protein) in the respiratory and olfactory mucosa, and in the bone marrow (femur) of male Fischer 344 rats exposed for 6 h to concentrations of 0.3, 2, 6, 10, or 15 ppm, 1 day following a single preexposure to the same concentration of unlabeled formaldehyde (Casanova-Schmitz et al., 1984). The major route of nucleic acid labeling at all concentrations and in all tissues was metabolic incorporation into respiratory mucosa (i.e., metabolism of formaldehyde with subsequent entry into the one-carbon pool). Protein labeling in the respiratory mucosa was mainly due to covalent binding at the higher formaldehyde concentrations. Most important with respect to the subject of this review was the fact that while the bone marrow was heavily labeled with ^{14}C, the highest concentrations were found in DNA, suggesting that one-carbon units derived from metabolism of [^{14}C]HCHO were being used for DNA synthesis. The $^{3}H/^{14}C$ ratios of proteins, DNA, and RNA from bone marrow were independent of administered formaldehyde concentrations, thereby demonstrating that inhaled formaldehyde did not form covalent adducts (e.g., DNA–protein cross-linking) with macromolecules in the bone marrow.

Casanova and Heck (1987) demonstrated that depletion of glutathione (GHS) in order to inhibit the metabolism of formaldehyde did not result in inhaled formaldehyde reaching the bone marrow. In this study, rats were treated with phorone, which mainly depletes GSH, followed by exposure to [^{3}H]- and [^{14}C]formaldehyde at concentrations up to 10 ppm. While there were significant increases in $^{3}H/^{14}C$ ratios of DNA, RNA, and proteins of the nasal respiratory mucosa relative to controls, suggesting decreased metabolism and increased covalent binding in these tissues, there was no increase in the $^{3}H/^{14}C$ ratios of bone marrow macromolecules relative to controls. Consequently, even when formaldehyde metabolism is inhibited by GSH depletion, there was no detectable covalent binding of [^{3}H]- and [^{14}C]formaldehyde to bone marrow macromolecules at formaldehyde levels used in this study.

In a study designed to assess immune function and host resistance, female B6C3F1 mice were exposed via inhalation to 15 ppm HCHO for 6 h/day for 21 days (Dean et al., 1984). Immune parameters examined related to potential hematopoietic toxicity included routine hematology, bone marrow (femur) cellularity, and CFU granulocyte–macrophage (GM) analysis. Bone marrow cellularity and clonogenic potential of bone marrow derived progenitor cells were not significantly different between exposed and controls. This study provides evidence that subchronic exposue to 15 ppm formaldehyde does not damage the bone marrow and is not likely a target organ for HCHO toxicity.

In contrast, a potential adverse effect of formaldehyde on the bone marrow was reported by Kitaeva et al. (1990). In this study, female Wistar rats were exposed via inhalation to low concentrations of formaldehyde (presumably 0.4 or 1.2 ppm), 4 h/day, 5 days/week, for 4 months. There was an increased incidence of chromosomal aberrations in bone marrow cells. However, this study, as reported, is difficult to interpret since key experimental procedures (e.g., dose levels) and statistical methods were not sufficiently described. Furthermore, the overwhelming majority of studies have not corroborated this finding, including some with considerably higher exposures. Therefore, this single study is not sufficient to demonstrate formaldehyde-induced bone marrow toxicity.

There are essentially no reported hematological effects following exposure of either humans or animals to formaldehyde. While accidental ingestion of a large quantity of formaldehyde was reported to cause an intravascular coagulopathy (Burkhart et al., 1990), several reports of human ingestion of lower doses have not shown any effects on the blood or blood-forming organs (Eells et al., 1981; Freestone and Bentley, 1989; Koppel et al., 1990). In animal studies, neither inhalation exposure (Appelman et al., 1988; Kamata et al., 1997; Kerns et al., 1983; Woustersen et al., 1987) nor oral exposure (Johannsen et al., 1986; Til et al., 1988; Tobe et al., 1989) to high doses of formaldehyde has produced any evidence of adverse hematological effects. One study in rats exposed to massive oral doses of formaldehyde (e.g., 80 mg/kg for 4 weeks) reported minor alterations in erythrocyte count and hemoglobin values (Vargova et al., 1993). As noted in ATSDR (1999), the lack of hematopoietic toxicity in these studies is "likely related to rapid metabolism prior to the formaldehyde reaching the blood and blood-forming components (bone marrow)." This has been confirmed in modeling predictions based on a three-dimensional, anatomically accurate computational fluid dynamics model of rat nasal airflow and inhaled gas uptake. When integrated with a physiologically based mathematical model incorporating tissue thickness, formaldehyde diffusion, and removal by enzymatic and nonenzymatic processes, the model predicted a rapid and highly nonlinear decline in formaldehyde concentrations in nasal tissues (Georgieva et al., 2005). The inability of exogenous formaldehyde to increase blood concentrations was also confirmed by Franks (2005) in a sophisticated mathematical model for the absorption and metabolism of formaldehyde vapor by humans. The results of this model demonstrated that following inhalation exposure, the increase in formaldehyde concentration in the blood was insignificant compared to existing endogenous levels. Therefore, confirmatory of experimental studies, these models suggest that it is highly unlikely that following inhalation formaldehyde would cause toxicity at sites other than the initial site of contact.

B. In Vitro and In Vivo Genotoxicity and Cytogenetic Effects

Formaldehyde is genotoxic in numerous systems, including bacteria (e.g., *Salmonella typhimurium, Escherichia coli*), fungi (e.g., *Saccharomyces cerevisiar, Neurospora crassa*), nematodes (e.g., *Caenorhabditis elegans*), fruit flies (*Drosophila melanogaster*), mouse lymphoma cells, and human lymphocytes (Ma and Harris, 1988). As noted by ATSDR (1999), "formaldehyde has displayed genotoxic activity in the majority of studies in a variety of in vivo tests with organisms ranging from

bacteria to rodents and a variety of in vitro tests including tests with cultured human cells. The weight of evidence indicates that formaldehyde itself is capable of directly reacting with DNA, and producing genotoxic effects, especially when metabolic capacities are exceeded." However, unanswered by any of these data is a central issue of this review, that is, do the genotoxic or cytogenetic effects of formaldehyde suggest or indicate a potential for bone marrow toxicity with subsequent progression to leukemia, particularly at doses that do not overtly overwhelm endogenous detoxification mechanisms?

For example, while an in vivo study with formaldehyde at an oral dose of 100 mg/kg reported positive effects in a mouse bone marrow micronucleus test and sister chromatid exchange (Pereira et al., 1982), lower in vivo doses (6.25 to 25 mg/kg ip) failed to produce these effects in femoral bone marrow examined for chromosomal aberrations and micronuclei (Natarajan et al., 1983). Clearly, it is possible to administer formaldehyde doses that can overwhelm or bypass detoxification mechanisms and make it to the bone marrow. However, as noted earlier, even following exposure of monkeys or rats to formaldehyde at doses of 6 and 14 ppm, respectively, blood concentrations of formaldehyde are not increased. This supports the hypothesis that at reasonably anticipated exposure levels of formaldehyde, the bone marrow would not be a site of toxicity.

There are studies that report the putative effects of formaldehyde on a variety of biomarkers, including lymphocyte DNA–protein cross-links (DPX), sister chromatid exchanges (SCE), chromosome aberrations (CA), and micronucleus assay (MN). For example, formaldehyde was reported to cause an in vitro and in vivo increase in DPX in human white blood cells taken from 12 workers exposed to formaldehyde and eight controls (Shaham et al., 1996). While there was a significant increase in DPX in white blood cells from exposed workers (anatomy department and pathology institute), the overlap with controls was notable. The increase could not be attributed to smoking, although the difference in DPC between smokers and nonsmokers appeared to be similar to the difference between exposed and nonexposed workers. The small sample limits the utility of these findings.

Shaham et al. (2002) measured SCE in peripheral lymphocytes of 90 workers from 14 hospital pathology departments who were occupationally exposed to formaldehyde and of 52 unexposed workers as controls. The SCE results were expressed as either the mean number of SCEs per chromosome or the proportion of high frequency cells (i.e., >8 SCEs), with a high correlation between these two variables. There was a significant difference between the adjusted means of both SCEs variables among the exposed group compared with that of the unexposed controls. Adjustment was made for age, sex, smoking habits, education workers, and origin. However, the significance of SCE is unknown and no prospective human study has validated this as a biomarker of human cancer risk of any type, including leukemia (Preston and Hoffman, 2001).

Suruda et al. (1993) prospectively investigated the effect of low-level exposure to formaldehyde on oral, nasal, and lymphoycte biological markers in a group of 29 mortician students who were about to take a course in embalming over an 85-day study period. Epithelial cells from the buccal area of the mouth and nose showed an increase in micronucleus frequency during the study period. In peripheral lymphocytes, the frequency of micronucleated lymphocytes significantly increased by 28%, while SCE decreased by 7.5%. There was a dose-response relationship between cumulative exposure and increases in buccal epithelial micronuclei in males, but not in females, and no dose-response relationship between changes in nasal cells and cumulative formaldehyde exposure for the entire study was reported. Additionally, there was also no correlation between cumulative formaldehyde exposure and changes in micronucleated lymphocytes. However, the significance of these findings is unknown and no prospective human study has validated micronuclei as a biomarker of human cancer risk of any type, including leukemia.

Numerous other studies have investigated the potential in vivo genotoxicity (i.e., SCE, CA, or DPX) in the peripheral lymphocytes of occupationally exposed workers compared to unexposed controls (Bauchinger and Schmid, 1985; He et al., 1998; Yager et al., 1986; Ying et al., 1997, 1999; Vasudeva and Anand, 1996; Thompson et al., 1984). As discussed later, the evidence that exposure to potentially carcinogenic chemicals is associated with an increase in SCE in peripheral lymphocytes is mixed. While these studies are of interest, the resulting data are frequently conflicting. The inability to link these markers to cancer risk of any type, particularly in a specific one target organ, is problematic for concluding that biomarkers measured in peripheral lymphocytes are indicative of an increase in leukemia risk Also, these markers are for circulating cells, and it has not been shown that these effects occur in stem cells that can transition to leukemia.

With respect to the central issue of whether chromosomal aberrations in peripheral lymphocytes from workers with occupational exposure to formaldehyde might be an indicator of potential hematopoietic risk, Dallas et al. (1992) conducted a cytogenetic analysis of lung (i.e., pulmonary lavage fluid) and bone marrow cells in rats after repeated exposure to formaldehyde. Male Sprague-Dawley rats were exposed to 0, 0.5, 3, or 15 ppm formaldehyde for 6 h/day, 5 days/week for 1 and 8 weeks. There was an increase in pulmonary lavage cells with CA after both 1 and 8 weeks of exposure with the greatest effect in animals exposed at 15 ppm for 8 weeks. However, there were no differences in the proportion of bone marrow cells with CA between animals exposed to formaldehyde and controls at either 1 or 8 weeks at any dose level.

The target organ specificity in pulmonary cells noted by Dallas et al. (1992) was confirmed in vitro with cultured bronchial epithelial and fibroblastic cells, where formaldehyde was shown to cause single-strand DNA breaks and DNA–protein cross-links (Casanova-Schmitz et al., 1984). In contrast, the lack of effects on bone marrow cells was demonstrated in an earlier study by Dallas et al. (1987) using flow cytometry to monitor the cell-cycle distribution of DNA and RNA in bone marrow and alveolar

macrophages in male Sprague-Dawley rats exposed to formaldehyde vapor concentrations of 0, 0.5, 3, or 15 ppm for 6 h/day, 5 days/week, for up to 24 weeks. While there were clear effects on pulmonary cells following all three doses, there were no formaldehyde-related effects on bone marrow cells at any dose or time point.

The data just described demonstrate that while formaldehyde can produce dose-related cytogenetic effects on some cells following direct exposure (i.e., bronchial epithelial cells), similar effects are not observed on cells distant from the site of administration such as bone marrow. This suggests that unless formaldehyde doses that grossly exceed metabolic capabilities are administered (e.g., 100 mg/kg), distant site toxicity (including bone marrow toxicity) is unlikely.

C. Formaldehyde and Cancer

Numerous studies in rodents have been conducted to determine the carcinogenic potential of formaldehyde. With the exception of one study (i.e., Soffritti et al., 1989, 2002, reviewed in detail later), no other studies have reported a carcinogenic effect other than at the site of administration, that is, nasal cancer in rats and mice following inhalation exposure and gastric cancer in rats following ingestion exposure. As noted by Nelson et al. (1986), "No evidence of toxicity was detected at sites other than the respiratory tract. Bone marrow hyperplasia present in the rat bioassay was not considered a primary effect of formaldehyde exposure, but secondary to anoxia due to the presence of obstructive masses in the nasal passages." A detailed review by Feron et al. (1991) noted that "Following inhalation exposure at levels causing cell damage and hyperproliferative changes in the epithelium of the nasal cavity, formaldehyde has been found to cause nasal cavity tumors (mainly squamous cell carcinomas) in rats (Kerns et al., 1983; Tobe et al., 1989; Sellakumar et al., 1985; Feron et al., 1989) and probably in mice (Kerns et al., 1983) but not in hamsters (Dalbey, 1982)." Since none of these studies reported any adverse effects on the bone marrow, they are not further reviewed here. In another inhalation study by Swenberg et al. (1980), formaldehyde was administered to rats at 0, 2, 6, or 15 ppm, 6 h/day, 5 days/week, for 18 months. In total, 43 tissues were examined and, as noted by the authors, "Compound-related lesions [squamous metaplasia] were restricted to the nasal cavity."

Til et al. (1989) conducted a 2-year drinking-water study of formaldehyde in Wistar rats. The mean HCHO doses administered to male and female animals were 0, 1.2, 15, or 82 mg/kg/day and 0. 1.8, 21, or 109 mg/kg/day, respectively. Treatment-related changes were only noted in the gastric mucosa, although there was no evidence of carcinogenicity either in the stomach or any other sites.

Of the many carcinogenicity studies on formaldehyde, the only one that has reported a carcinogenic effect at a site distant from the point of administration (i.e., nasal passages or gastric mucosa) was by Soffritti et al. (1989). In this study, male and female Sprague-Dawley rats of different ages (i.e., 7 weeks old at start, 25 weeks old at start [i.e., breeders] and 12-day embryos [i.e., in utero exposure]) were exposed to formaldehyde in drinking water at concentrations of 0, 10, 50, 100, 500, 1000, 1500, and 2500 mg/L for up to 104 weeks. Only the 7-week-old rats were exposed to graded doses of formaldehyde (i.e., 10–1500 mg/L), while the 25-week-old and in utero rats were only exposed to formaldehyde at either 0 or 2500 mg/L. In one of the "control" groups, methyl alcohol was added to the drinking water at a concentration of 15 mg/L, although there was no explanation for why this was done. Histopathology examinations were conducted on most tissues, including the femur. As reported by Soffritti et al. (1989), there was an increase in "lymphoblastic leukemias and lymphosarcomas" and "immunoblastic lymphosarcoma." While these findings were increased at doses >500 mg/L, the lack of any statistical analysis of the data precludes the ability to accurately assess the data; for example, the reported incidence of "immunoblastic lymphosarcoma" did not appear to be dose related, and "other leukemia" appeared similar in exposed and controls. There did not appear to be any differences between male and female breeder rats and controls with respect to the various leukemias reported, although again, the absence of statistical analysis makes an accurate assessment of these data impossible. Additionally, while bone marrow was one of the tissues specifically mentioned as part of routine histopathology, there was no mention of findings from this tissue. Because of the numerous questions concerning the conduct of this study, it is difficult to judge the findings in context with other data. As noted by Feron et al. (1990, 1991), none of the contradictory findings from other oral dosing studies that were available when Soffritti et al. (1989) published their results were discussed. In addition, while Soffritti et al. present their historical control data for stomach, intestine, and gastrointestinal (GI) neoplasms in Sprague-Dawley rats, historical control data for lymphoblastic leukemia-lymphosarcoma are not presented. As described by Feron et al. (1990, 1991), historical untreated control data in Sprague-Dawley rats of the colony used show that the incidence of leukemia varies widely, with reported spontaneous incidence rates similar to those reported by Soffritti et al., suggesting that treatment-related effects may have been unrelated to formaldehyde exposure. As concluded by Feron et al. (1991), "Since, however, crucial information on procedures and histopathology of non-neoplastic changes is lacking, the adequacy of this study and the relevance of the data can hardly be judged, if at all." In reviewing the results of Soffritti et al. (1989), ATSDR (1999) expressed skepticism: "Another limitation to the strength of the evidence for formaldehyde-induced leukemia is the lack of a consistent dose-response relationship in the Soffritti et al. study.... The second part of the Soffriti et al. (1989) study found no statistically increased incidence of leukemia in groups of breeding pairs of rats or their offspring exposed for life to the higher dose level of 313 mg/kg/day. A further limitation is the absence of corroborating evidence for effects at sites distant from portals-of-entry in the other drinking water rat studies, and in inhalation-exposure animal studies." The Cancer

Assessment Committee of the Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration (FDA), also reviewed the study of Soffritti et al. (1989), concluding that the data reported were "unreliable" due to "a lack of critical detail . . . questionable histopathological conclusions, and the use of unusual nomenclature to describe the tumors." Consequently, the FDA "determined that there is no basis to conclude that formaldehyde is a carcinogen when ingested" (U.S. FDA, 1998). Finally, Soffritti et al. (2002) again reported the results first published as Soffritti et al. (1989). This appeared to be the same study except that the reported incidence of leukemia was almost doubled in most treatment groups, that is, 45 versus 91 in males and 34 versus 60 in females. However, information on historical control incidences of leukemia was still lacking and there was no explanation for the dramatic changes in the incidence of leukemia in the two reports.

The ability of formaldehyde to cause leukemia in animals exposed either by inhalation or ingestion must be judged in the context of all available data. Of the numerous long-term carcinogenicity studies, including exposure by inhalation or via drinking water, that have investigated the carcinogenic potential of formaldehyde, only one (i.e., Soffritti et al., 1989, 2002) has reported an increased incidence of leukemia. Leukemia was not reported in any other of seven inhalation bioassays with formaldehyde, nor was it detected in three other drinking-water studies in which rats were exposed to doses as high as 1.9 g/L or 5 g/L (Takahashi et al., 1986; Tobe et al., 1989; Til et al., 1989). As enumerated earlier, given the limitations and inconsistencies as reported by the Soffritti et al. (1989, 2002) study, it is difficult to reconcile the reported findings of leukemia with the rest of the peer-reviewed literature.

VI. CONCLUSIONS

The data on benzene and several classes of cancer chemotherapeutic drugs demonstrate a sequence of events that must occur prior to the development of leukemia in either animals or humans. First there must be evidence that a particular suspect leukemogen can reach the bone marrow following exposure. Second, there needs to be a demonstrable toxic effect on bone marrow cells that is related to leukemia pathways. Third, current models of leukemogenesis indicate that the leukemogen must be genotoxic. These key fundamental aspects of the mode of action for leukemogenic substances, such as benzene and some cancer therapeutic drugs, are simply not fulfilled by the available data on formaldehyde. With the exception of substantial exposure that is unlikely to be present in the human setting where epidemiological studies have been conducted, there is no evidence to suggest that formaldehyde reaches any target organ beyond the site of administration, such as the bone marrow. Furthermore, with the same caveat, there is no indication that formaldehyde is toxic to the bone marrow/hematopoietic system in the in vitro studies. Finally, any theory or hypothesis that formaldehyde might be capable of causing leukemia via a mode of action different from the above noted sequence of events (e.g., mutation of circulating stem cells with subsequent transport to the bone marrow) should be capable of being experimentally validated. An inability to do this precludes support for this hypothesis and remains speculative. In this regard it is worthwhile to note that rats have bone marrow stem cells that move into and out of the circulation. It is therefore reasonable to expect that such stem cells could be "mutated" as blood flowed through the lungs with subsequent transport back to the bone marrow in the numerous inhalation bioassays with formaldehyde. The lack of leukemia or any evidence of bone marrow toxicity in any of these studies suggests that this hypothesized sequence of events does not occur.

The underlying biology of leukemogenesis as just outlined is also corroborated in an extensive review prepared by the National Center for Environmental Assessment of the lymphoid and hematopoietic diseases induced in humans and rodents following exposure to chemical agents known to be associated with leukemogenesis (U.S. EPA/NCEA, 1997). Included are the same chemicals used in the present review, i.e., benzene, alkylating agents and topoisomerase inhibitors. In addition to confirming the necessity of the bone marrow as a target organ for leukemogenesis, the conclusions also amplify the findings of the present review:

> "By evaluating the characteristics of known leukemia-inducing agents, a number of generalizations appear to be warranted. (1) The primary type of lymphohematopoietic cancer induced by chemicals and radiation in humans is myeloid leukemia (ANLL). . . . (2) Potent human leukemia-inducing agents induce significant myelotoxicity in structural chromosomal aberrations in exposed humans. Similar effects are seen when these agents are administered to animal models. (3) Administration of human leukemia-inducing agents to mice results in increases in lymphohematopoietic tumors. However, in contrast to the human, these tumors are primarily lymphoid in origin. (4) The rat is considerably less responsive than the mouse for induction of lymphohematopoietic neoplasia following administration of human leukemogens. However, the resulting neoplasms in the rat are also are primarily lymphoid in origin."

It should be emphasized that none of the numerous valid carcinogenicity studies in rats or mice reported any effects on lymphoid tissue as a consequence of exposure to formaldehyde.

As already described, several studies have reported associations between formaldehyde and biomarkers of exposure such as DPX, SCE, CA, and MN in peripheral lymphocytes. With the exception of CA, where only some data exists, there is insufficient evidence to conclude that an increase in these other markers predicts an increased future risk. Most investigations have studied chromosomal aberrations (CA), because it is generally accepted that chromosomal mutations are causal events in the development of cancer. However, as noted later, while some studies have reported an increased risk of total cancers, it has never been proven that increased chromosomal damage is associated with excess cancer risk of a particular disease. Two additional techniques, SCE and MN, have also been used, although the toxicological or clinical significance of these latter two methods is not fully understood (Hagmar et al., 1998a, 1998b, 2001, 2004). For example, in a pooled analysis of occupational

cohorts, 3541 subjects were examined for CA, 2703 for SCE, and 1496 for MN. While there was a significantly elevated risk of all cancer combined among subjects with high CA frequency, this was not observed for those with medium or low CA frequency. There was no association between the SCE or MN frequencies and subsequent cancer incidence/mortality. Of particular interest was the finding that the risk for high versus low levels of CA was similar in subjects heavily exposed to carcinogens and in those who had never, to their knowledge, been exposed to any carcinogenic chemicals during their lifetime. In a similar study, the risk for high versus low levels of CA was similar in subjects heavily exposed to carcinogens and in those who had never been exposed to any carcinogenic chemicals during their lifetime, once again supporting the idea that chromosome damage itself is involved in the pathway to cancer (Bonassi et al., 2000).

While chromosome damage is likely involved in the pathway to cancer, based on this kind of evidence alone, it cannot be concluded that exposure to particular chemicals is responsible for specific kinds of cancer. This view is corroborated by Preston and Hoffmann (2001), who note that "individuals with higher frequencies of chromosome aberrations for whatever reason (genetic or environmental) are as a group at greater risk of dying from cancer. This is very different from concluding that exposures to mutagens that result in a higher frequency of chromosome aberrations in peripheral lymphocytes leads to an increase risk of cancer, especially for specific tumor types." While benzene has also been reported to cause CA in peripheral lymphocytes, this is not the evidence on which the established leukemogenic potential of benzene is based. Rather, benzene was first associated with AML in humans, has documented bone marrow toxicity in humans and animals, and has also been shown to cause leukemia in rodents. Thus, although it might be hypothesized that finding CA in the peripheral lymphocytes of benzene-exposed workers is a risk factor for the subsequent development of AML, it is the antecedent knowledge that corroborates this hypothesis. There are no animal studies that report an increased rate of CA with formaldehyde exposure and the few human studies are conflicting (e.g., Thomson et al., 1984; Vasudeva and Anand, 1996; Ji-Liang et al., 1998). However, none of these data can be interpreted as indicating an increased risk of cancer, including leukemia. Thus, the limited evidence for genotoxicity in humans does not provide sufficient evidence to be corroborative of human epidemiology studies. In this regard, it is worthwhile to note that the alkylating agent methotrexate is well established as producing multiple chromosomal abnormalities in human lymphocytes both in vitro and in vivo (Mondello et al., 1984; IARC, 1987). However, after many years of observation on thousands of patients with rheumatoid arthritis, lupus, psoriasis, and various malignancies treated with methotrexate, there is no evidence of an increase risk of s-AML following prolonged use. This observation calls into question the value of citing lymphocyte chromosomal aberrations as predictive of a particular chemical's leukemogenic effect in humans.

As reviewed by Heck and Casanova (2004) as well as in this review, formaldehyde does not cause DPX or CA in bone marrow cells. This may be an important mechanistic consideration if, as described by Conolly et al. (2004), DPX as a precursor event (i.e., either descriptive or etiologic) in formaldehyde-induced nasal squamous-cell carcinoma would be similarly a precursor event in formaldehyde-induced leukemia. This would necessarily require a demonstration of formaldehyde-induced DPX in the bone marrow and not just in circulating lymphocytes as reviewed above. While it is not known if DPX is etiologically implicated in formaldehyde-induced nasal cancer, it appears to be a useful surrogate for modeling the genotoxic and cytolethality/regenerative cellular proliferation potential of formaldehyde (Conolly et al., 2004). The inability of formaldehyde to induce DPX in bone marrow would further support the biological implausibility of formaldehyde-induced leukemia.

The final corroboration demonstrating the biological plausibility of leukemogenesis is the ability of leukemogenic substances to actually cause the development of leukemia. Benzene and the cancer chemotherapeutic drugs considered in this review clearly fulfill this criterion by their demonstrated ability to cause leukemia in animal models. As shown by the totality of the animal carcinogenicity data on formaldehyde, there is no credible scientific evidence that exposure is capable of causing leukemia. Of the numerous inhalation or drinking-water studies on formaldehyde, all are unequivocally negative with respect to demonstrating a leukemogenic effect. Only one study (i.e., Soffritti et al., 1989, 2002) reported leukemia in rats following drinking water exposure to formaldehyde. As detailed in this review, due to the numerous deficiencies in the conduct and interpretation of this study, the results can be discounted in the context of the totality of the database.

With respect to the central theme of this review (i.e., an evaluation of the biological plausibility that formaldehyde might be leukemogenic), all of the substances considered have been associated with leukemia in humans and have also demonstrated hematopoietic toxicity and leukemia in animal models. In other words, the biological plausibility of demonstrated leukmogenesis in humans has been confirmed in animal studies and augmented by additional in vitro or in vivo data, particularly data demonstrating bone marrow toxicity. The data on benzene, the alkylating agents considered in this review (plus a few others), topoisomerase inhibitors, and radiation are summarized in Table 1.

In summary, as described in this review, as well as in the review by Heck and Casanova (2004), an extensive database demonstrates that (1) normal metabolic processes prevent formaldehyde from entering the systemic circulation, (2) the bone marrow is not a target organ for formaldehyde toxicity, (3) formaldehyde does not cause leukemia in animal studies, and (4) to the extent that formaldehyde produces cytogenetic effects in lymphocytes from exposed workers, these findings have unknown significance to the development of any particular kind

TABLE 1
Comparative data on in vivo/in vitro bone marrow/hematopoietic toxicity and leukemia induction in animals and humans of leukemogenic chemicals and formaldehyde

Substance	In vivo effects on marrow or hemato poiesis	In vitro effects on marrow or hemato poiesis	Mutagenic or genotoxic	Leukemia in animals	Leukemia in humans
Benzene	+++	+++	++	+++	+++
Cyclophosphamide[b]	+++	+++	+++	+++	+++
Myeleran[b]	+++	+++	+++	+++	+++
Chlorambucil[b]	+++	+++	+++	+++	+++
Procarbazine[b]	+++	+	+++	+++	++
Thiotepa[b]	++	+	+++	+	++
Etoposide[c]	++	++	+	+	+++
Teniposide[c]	++	++	+	+	+++
Doxorubicin[c]	++	+	+++	ND	+++
X and γ radiation	+++	++	+++	Yes	Yes
Formaldehyde	No[a]	No[a]	++	No[a]	?

Note. Adapted from IARC (1981, 1987), U.S. EPA/NCEA (1997), and other cited references. +++ = Strong unambiguous; ++ = less strong; + = weak, equivocal; ? = questionable; ND = no data; NE = nonexistent.

[a] See later discussion on formaldehyde.

[b] Alkylating agent.

[c] Topoisomerase inhibitor.

of cancer, including leukemia. Collectively, these data fail to corroborate the epidemiology results.

In today's regulatory climate, there is an increased emphasis on understanding the mode of action of chemical carcinogenesis as a confirmation of biological plausibility. This concept is explicitly recognized in the U.S. EPA (2005) recently finalized cancer risk assessment guidelines (e.g., "An inference of causality tends to be strengthened by consistency with data from experimental studies or other sources demonstrating plausible biological mechanisms"). Particularly with respect to the possibility that exposure to formaldehyde might be etiologically associated with leukemia, the U.S. EPA (2005) guidelines note that "It is important that the hypothesized mode of action and the events that are part of it be based on current understanding of the biology of cancer to be accepted. If the body of information under scrutiny is consistent with other examples (including structurally related agents) for which the hypothesized mode of action is accepted, the case is strengthened." The position of the International Programme on Chemical Safety (IPCS, 1999) on this issue is virtually identical. Based on the epidemiological data, it is reasonable to expect that if formaldehyde was capable of inducing leukemia in exposed workers then the abundant in vivo and in vitro data on this chemical would offer some supporting evidence of the biological plausibility of this effect consistent with the leukemogenic chemicals discussed in this review. However, based on an understanding of the biological events involved in the process of chemical leukemogenesis, it is biologically implausible that formaldehyde exposure is capable of inducing leukemia in animals or humans. This conclusion is further supported by the in-depth review by Heck and Casanova (2004), who observed that "the abundance of negative evidence . . . is undisputed and strongly suggests that there is no delivery of inhaled formaldehyde to distant sites. Combined with the fact that formaldehyde naturally occurs throughout the body, and that multiple inhalation bioassays have not induced leukemia in animals, the negative findings provide convincing evidence that formaldehyde is not leukemogenic."

The lack of relevant mode of action data on formaldehyde when compared to the proven leukemogenic substances described in this review does not support a conclusion that it is biologically plausible that formaldehyde is capable of causing leukemia in animals, much less in humans. Consequently, there are insufficient laboratory data to conclude that there is a biologically plausible relationship between formaldehyde exposure and leukemia risk.

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